AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claim 1 (currently amended): A recrystallization inhibition method for determining the presence, relative concentration, and or [~~and/or~~] activity of thermal hysteresis proteins comprising:

providing a proteinaceous composition in a solvent to form a test solution;

flash freezing said solution;

raising the temperature of the frozen solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within said solution;

maintaining said frozen solution at the annealing temperature for a length of time sufficient to allow for ice recrystallization;

monitoring the ice crystal grain size changes over time; and

determining the presence of functional thermal hysteresis proteins in said solution given the retention of significantly smaller ice crystal grain sizes relative to at least one control solution.

Claim 2 (original): The recrystallization inhibition method as defined in claim 1, wherein said solvent selected from the group consisting of water, saline, PBS, or other isoosmotic inorganic or organic solutions.

Claim 3 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein two or more control solutions are used; where one control is said [~~the~~] solvent and the other is a control solution for non-specific recrystallization inhibition effects.

Claim 4 (original): The recrystallization inhibition method as defined in claim 1, whereby the proteinaceous composition is a thermal hysteresis protein with a known activity.

Claim 5 (original): The recrystallization inhibition method as defined in claim 1, wherein said proteinaceous composition is purified Tm 12.86. or Tm 12.84.

Claim 6 (original): The recrystallization inhibition method as defined in claim 1, wherein said proteinaceous composition is selected from the group consisting of antifreeze polypeptides, antifreeze glycopeptides, recombinant antifreeze polypeptides, recombinant antifreeze glycopeptides, synthetic antifreeze polypeptides analogs, synthetic antifreeze glycopeptide analogs, cell culture products, activators, recombinant bacterial products, recombinant products, uncharacterized plant products and transgenic plant products.

Claim 7 (original): The recrystallization inhibition method as defined in claim 1, wherein said proteinaceous composition has unknown functional antifreeze protein activity.

Claim 8 (currently amended): The recrystallization inhibition method as defined in claim 4 wherein said proteinaceous composition [~~of~~] includes Tm 12.86 [~~is~~] present between 0.5 ug/ml [~~ug~~] to 25 ug/ml.

Claim 9 (currently amended): The recrystallization inhibition method as defined in claim 2, wherein the [~~said~~] protein content in said proteinaceous composition is less than or equal to 1 mg/ml in saline and PBS; and less than or equal to 0.005 mg/ml in water.

Claim 10 (original): The recrystallization inhibition method as defined in claim 1, under conditions to eliminate non-thermal hysteresis protein induced recrystallization inhibition effects.

Claim 11 (original): The recrystallization inhibition method as defined in claim 10, wherein said conditions in saline are at -6 (C for 30 min with total protein content less than or equal to 1 mg/ml; or in water at -2 (C for 2 hours with total protein content less than or equal to 0.005 mg/ml.

Claim 12 (original): The recrystallization inhibition method as defined in claim 10, under conditions to avoid hyperosmotic solutions.

Claim 13 (original): The recrystallization inhibition method as defined in claim 1, wherein monitoring of ice crystal grain size changes over time is by photomicroscopy, digital or video imaging.

Claim 14 (original): The recrystallization inhibition method as defined in claim 1, wherein quantitative data is collected by measurement of the mean largest ice grain size for both said test and control solutions to provide a basis for numerical assessment of the extent of recrystallization inhibition occurring.

Claim 15 (original): The recrystallization inhibition method as defined in claim 14, wherein composite mlgs are obtained for said test solution and said control solution; which are then statistically compared.

Claim 16 (original): The recrystallization inhibition method as defined in claim 1, wherein quantitative data collection is collected by assessment using a densitometer of light transmitted through a low magnification full view photographic negative of frozen sample wafer; absorbance peaks for said test solution is evaluated for maximum amplitude and statistically compared with said control solution.

Claim 17 (original): The recrystallization inhibition method as defined in claim 1, wherein a dilution profile of said test solution is obtained over a wide dilution range until mlgs, or another quantifiably assessed response variable, are no longer significantly different from the saline/PBS/ and/or non-THP containing proteinaceous control solutions.

Claim 18 (original) The recrystallization inhibition method as defined in claim 17, wherein composite mlgs, or absorbance peak area (light scattering), or computer generated units (digital/video imaging)) are calculated for said test solution and plotted as a function of the logarithm of sample concentration, with replicate dilution series tested, and compared to control solution
baseline.

Claim 19 (currently amended): The recrystallization inhibition method as defined in claim 17, wherein linear

regression analyses is used to approximate the linear portion of the dilution profile, with application of a transforming function [arcsine[(mlgs)0.5] verses log(dilution)] ~~[[arcsine[(mlgs)0.5] verses log(dilution)]]~~ to mlgs to limit inherent curvature of dilution plots caused by the "leveling off" of mlgs values for both very dilute and very concentrated thermal hysteresis protein samples.

Claim 20 (original): The recrystallization inhibition method as defined in claim 1, wherein linear regression analyses provides the basis for development of a numerical factor (RI factor) describing the activity of the test solution with respect to recrystallization inhibition capability.

Claim 21 (original): The recrystallization inhibition method as defined in claim 20, wherein the RI factor is equal to the absolute value of the logarithm of the minimum test solution dilution required to eliminate recrystallization inhibition activity.

Claim 22 (original): The recrystallization inhibition method as defined in claim 21, wherein the RI factor is a measure of test solution recrystallization inhibition strength, according to the assessed exponential factor required for sufficient dilution of test solution to lose recrystallization inhibition activity, and providing a relative assessment of functional thermal hysteresis concentration within said test solution.

Claim 23 (original): The recrystallization inhibition method as defined in claim 21, wherein the RI factor provides a relative assessment of functional thermal hysteresis protein concentration, and comparisons of various test solutions concentrations given translational shifts along the X axis.

Claim 24 (original): The recrystallization inhibition method as defined in claim 19, wherein the regression line slope and Y-intercept reflect the recrystallization inhibition potency of a given test solution, thermal hysteresis protein species, recombinant thermal hysteresis protein product, synthetic thermal hysteresis analogue, or the like.

Claim 25 (original): The recrystallization inhibition method as defined in claim 19, wherein slope comparisons and shifts along Y-intercept provide relative potency comparisons between test solutions, thermal hysteresis species and the like.

Claim 26 (original): The recrystallization inhibition method as defined in claim 20, wherein expected concentrations of Tm 12.86 producing equivalent RI profiles are deduced, and provide reference interpretations of the test solution(s)' functional activity(ies) to an antifreeze protein of known characterized parameters experimentally measured.

Claim 27 (original): The recrystallization inhibition method as defined in claim 22, wherein activity and potency of said test solution may include a combination of more than one type of thermal hysteresis protein, and/or thermal hysteresis protein plus activator solutions such as in test solution of hemolymph, or artificial solutions containing known amounts of purified thermal hysteresis protein with an activator supplement.

Claim 28 (original): The recrystallization inhibition method as defined in claim 1, further comprising mathematical modeling of the recrystallization inhibition process with prediction of effects on slope and Y-intercept and log/log transformations for test solution mlgs data and analysis.

Claim 29 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein the relationship between RI factors and thermal hysteresis levels for functionally active test solutions are described by the equation: RI factor = 1.428 LOG(TH) + 3.703.

Claim 30 (original): The recrystallization method as defined in claim 1, wherein a random sampling method is used for data collection generating mlgs which significantly eliminates the impact of intrasample ice crystal grain heterogeneity at high annealing temperature and with saline/PBS solvents.

Claim 31 (original): The recrystallization inhibition method as defined in claim 1, further used for concurrent multiple sample testing of solutions.

Claim 32 (original): The recrystallization inhibition method as defined in claim 31, wherein said multiple testing of solutions includes the "sandwich" method; and application via a 96 well plate device.